

AWARD NUMBER: W81XWH-14-1-0529

TITLE: Identifying DNA Methylation Features that Underlie Prostate Cancer Disparities

PRINCIPAL INVESTIGATOR: Brandon Pierce

RECIPIENT: The University of Chicago
Chicago, IL 60637

REPORT DATE: October 2017

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release; Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202.					
1. REPORT DATE October 2017		2. REPORT TYPE Annual		3. DATES COVERED 30 Sep 2016 - 29 Sep 2017	
4. TITLE AND SUBTITLE Identifying DNA Methylation Features that Underlie Prostate Cancer Disparities				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-14-1-0529	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Brandon Pierce F-Mail:bpierce@health.bsd.uchicago.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) UNIVERSITY OF CHICAGO, 5801 S ELLIS AVECHICAGO IL 60637-5418				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT In the U.S., African Americans (AA) are more likely to be diagnosed with prostate cancer than European American (EA), and after diagnosis, AA men are more likely to die from prostate cancer than EA men. We hypothesize that differences in DNA methylation patterns across ethnic groups may contribute to prostate cancer disparities. Our objective is to conduct a genome-wide study of methylation patterns in prostate tumors and adjacent normal tissue derived from both AA and EA individuals. We will determine if DNA methylation patterns in prostate tissue (both cancerous and normal tissue) differ between AA and EA individuals. We will also identify methylation features that differ between tumor and normal tissue. Using this information, we can then determine if methylation events that accompany prostate cancer development differ between ethnic groups. In addition, we will attempt to determine if these epigenetic differences are driven by genetic and environmental factors that vary by ethnicity. Developing an understanding of these differences is a critical and necessary step towards understanding and addressing prostate cancer disparities. Features identified here can be used in future studies of disparities to better characterize the prostate cancer phenotype in diverse populations.					
15. SUBJECT TERMS None listed					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	16	19b. TELEPHONE NUMBER (include area code)

TABLE OF CONTENTS

	<u>Page</u>
1. Introduction	4
2. Keywords	4
3. Accomplishments	4
4. Impact	6-7
5. Changes/Problems	7-9
6. Products	9-11
7. Participants & Other Collaborating Organizations	12-13
8. Special Reporting Requirements	13-14
9. Appendices	14

1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

In the U.S., there are pronounced racial disparities in prostate cancer incidence and mortality. We hypothesize that the methylation features in prostate tissue (both cancerous and normal) differ between AA and EA men and that the methylation events that accompany prostate cancer development differ between ethnic groups. Our goals are too identify methylation features that vary by ethnicity and to identify ethnicity-specific methylation features of prostate cancer that could contribute the racial disparities that exist in the U.S.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Disparities, prostate cancer, DNA methylation, SNP

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Process Aims: Generate genome-wide methylation and SNP Profiles
Primary Aim #1: Determine if methylation profiles differ by race/ancestry
Primary Aim #2: Identify ethnicity-specific markers of prostate cancer
Primary Aim #3: Identify methylation Quantitative Trait Loci

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Process Aims: As described in our prior progress report, we have collected all of the questionnaire data and bio-specimens we need to complete our aims. Our recruitment efforts are complete; we have approached > 250 patients and enrolled >200 patients (as of December 2017). Participant characteristics are described in **Table 1**.

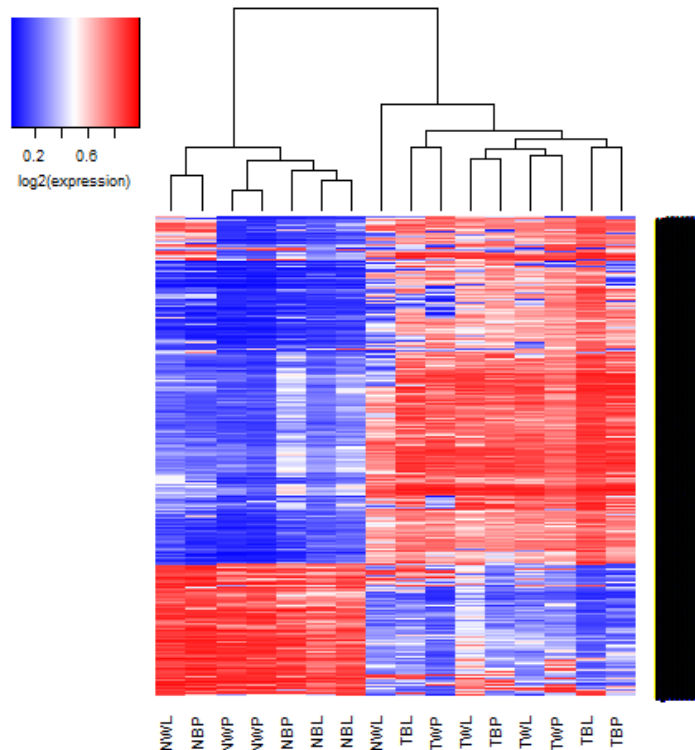
Table 1. Prostate Cancer Patient characteristics

Patient Characteristics		African American (n= 101)	European American (n= 111)
Age	Years	65 (8.4)	66 (7.2)
Education	≤ High School	15 (14.9%)	13 (11.7%)
	Some college	29 (28.7%)	22 (19.8%)
	≥ College degree	21 (20.8%)	56 (50.5%)
	NA	36 (35.6%)	20 (18%)
BMI (kg/m²)	Normal (18.5-24.9)	4 (4%)	11 (10%)
	Overweight (25-29.9)	23 (22.8%)	41 (36.9%)
	Obese (> 30)	35 (34.6%)	40 (36%)
	NA	39 (38.6%)	19 (17.1%)
Smoking	Never	30 (29.7%)	53 (47.7%)
	Former	24 (23.8%)	35 (31.5%)
	Current	7 (6.9%)	1 (1%)
	NA	40 (39.6%)	22 (19.8%)
Family History of Prostate Ca	Yes	18 (17.8%)	20 (18%)
	No	48 (47.5%)	73 (65.8%)
	NA	35 (34.7%)	18 (16.2%)
Gleason Score	≤6	9 (8.9%)	3 (2.7%)
	7(3+4)	60 (59.4%)	59 (53.2%)
	7(4+3)	17 (16.8%)	26 (23.4%)
	≥8	9 (8.9%)	17 (15.3%)
	NA	6 (6%)	6 (5.4%)
Prostate-specific Antigen (PSA)	ng/mL	76.7 (42.9)	78.2 (40.7)
pTNM Stage	T2	68 (67.3%)	48 (43.2%)
	T3	29 (28.7%)	58 (52.3%)
	NA	4 (4%)	5 (4.5%)

We have now obtained, stained, and dissected all bio-specimens needed for this project (*Tasks 1 and 2 from SOW*). We have extracted DNA samples for 75 African American (AA) patients and all 75 European American (EA) patients (for both prostate tumor and nearby “normal” tissue) as proposed in our aims. Based on a qPCR-based assay for evaluating DNA integrity, all DNA samples pass QC for use of the Illumina FFPE Restoration kit (*Task 3*). In this period, we generated DNA methylation data for a pilot batch of 16 samples, and the data quality was excellent, with the vast majority of CpGs (>99%) successfully detected (*Task 4, partial*). All DNA samples will be delivered to the Genomics Core Facility at the University of Chicago in the coming weeks to generate the remaining methylation and SNP data as proposed in the aims and perform data processing and cleaning (*Task 5*).

Primary Aim 1: We conducted preliminary analyses of genome-wide DNA methylation data for a small set of DNA samples (n=16) obtained from cancer and normal tissue from both AA and EA individuals. Data was processed and cleaned by the Genomics Core Facility at the University of Chicago. We conducted cluster analyses to ensure our data was clustering by cancer vs. normal status, and the patterns are general as expected (**Figure 1**). One normal sample clusters between tumor and normal, suggesting potential contamination with cancer cells. Among normal samples, one AA sample clusters with EA, suggesting admixture with substantial European ancestry. The remaining tasks under this aim will be performed once the full dataset (n=150) has been assembled.

Figure 1. Hierarchical clustering using the 5,000 most variable CpG sites distinguishing tumor from normal. Red corresponds to higher and blue to lower methylation. The “log2(expression)” corresponds to the methylation beta-value (range: 0 to 1). N/T: normal/tumor; W/B: white/black; L/P: laser-capture/punch.



Primary Aims 1- 3: Aim 1-3 entail various analyses of the full dataset (n=150) that we are still in the process of generating. These will be conducted after the full data set is generated, so these aims have not yet been addressed.

What opportunities for training and professional development has the project provided?

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. “Training” activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. “Professional development” activities result in increased knowledge or skill in one’s area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to Report

How were the results disseminated to communities of interest?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?

If this is the final report, state “Nothing to Report.”

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We will continue to generate the rest of the DNA methylation data and SNP data for the full set of patients we have recruited, as proposed in the aims. We will conduct all analyses proposed in aims 1-3 using the full set of DNA samples.

- 4. IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to Report

What was the impact on other disciplines?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report

What was the impact on technology transfer?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report

What was the impact on society beyond science and technology?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report

- 5. CHANGES/PROBLEMS:** The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, “Nothing to Report,” if applicable:

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

No major problems to report. Our data generation and statistical analyses are behind schedule due to delays with patient recruitment experienced in year 1 (as described in our prior progress report).

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

We did not begin laboratory activities in year 1 due to recruiting delays. Thus, the funds budgeted for years 1-3 lab work have been (and will be) spent in years 2-3 and the no-cost extension period.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state “Nothing to Report.”

- **Publications, conference papers, and presentations**

Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume; year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to Report

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to Report

- **Inventions, patent applications, and/or licenses**

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate “no change”.

Example:

Name: Mary Smith
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID): 1234567
Nearest person month worked: 5

Contribution to Project: Ms. Smith has performed work in the area of combined error-control and constrained coding.
Funding Support: The Ford Foundation (Complete only if the funding support is provided from other than this award.)

Name: Brandon Pierce	No Change
Name: Karen Kim	No Change
Name: Donald Vander Griend	No Change
Name: Lin Chen	No Change
Name: Marc Gillard	No Change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Brandon Pierce:

New

R35 ES028379

NIH/NIEHS

09/15/17-07/31/25

Arsenic and the Human Genome: susceptibility and response to exposure

*Project also includes Lin Chen.

Closed

R01 ES020506

NIH/NIEHS

A Study of Telomeres in an Arsenic-Exposed Bangladeshi Cohort

09/24/11-06/30/17

Donald Vander Griend

New

R21 DK110644

NIH

Bomb Pulse Carbon-14 Dating of Prostate Tissues to Elucidate the Origins of BPH

08/01/17-07/31/19

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:

Organization Name:

Location of Organization: (if foreign location list country)

Partner’s contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner’s facilities for project activities);*
- *Collaboration (e.g., partner’s staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner’s staff use each other’s facilities, work at each other’s site); and*
- *Other.*

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to <https://ers.amedd.army.mil> for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on <https://www.usamraa.army.mil>) should be updated and submitted with attachments.

9. **APPENDICES:** Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.